Morphological and physiological responses of beech (*Fagus sylvatica* L.) seedlings to grass-induced belowground competition

Lluis Coll*[a], Philippe Balandier[a], Catherine Picon-Cochard[b]

[a] CEMAGREF – U.R. DFCF, Applied Ecology of Woodlands Research Team

24, av. des Landais, BP 50085, 63172 Aubière cedex, France.

Tel : +33-473-440-732 Fax : +33 473-440-698 E-mail : lluis.coll@clermont.cemagref.fr

[b] INRA – U.R. Agronomie, 234 av. du Brezet, 63039 Clermont-Ferrand, France.

Running head : Beech-grass belowground competition

Summary

We examined the morphological and physiological response of beech (*Fagus sylvatica* L.) seedlings to grass-induced belowground competition in full light conditions. Two-year-old beech seedlings were grown during two growing seasons in 160 l containers (*i*) in bare soil conditions and (*ii*) with a mixture of five grasses widely represented in semi-natural meadows of central France. At the end of the second growing season, beech growing with grass presented significant reductions on diameter and height growth, annual shoot elongation and stem, root and leaf biomasses, but the root to shoot biomass ratio increased in such conditions. Grasses highly reduced soil water availability which was positively correlated with daily seedling diameter increment. Beech seemed to respond to water deficit by anticipating stomatal closure. Evidence of nitrogen competition by grasses was found, but its effect on seedlings development could not be separated from that derived from water competition. By labelling the plants with ^{15}N we showed that nitrogen absorption by beech seedlings was very low when growing with grasses, since grasses took up more than 97% of the total nitrogen absorbed in the container.

We conclude that, even if beech presents signs of morphological and physiological adaptation to belowground competition, beech development in full light conditions may be strongly restricted by the high competitiveness of typical full light grass species.

Keywords: Fagus sylvatica, competition, soil water content, leaf-gas exchange, ^{15}N, seedling architecture

Introduction

Beech (*Fagus sylvatica* L.) is one of the major broad-leaved tree species in forests of Central and Western Europe. Since beech is considered a shade-tolerant species (Ellenberg 1988), beech regeneration and development under different moderate light regimes have been widely studied (Gemmel et al. 1996, Le Goff and Ottorini 1999, Collet et al. 2001). Beech responds to different light environments in terms of morphological (Nicolini and Caraglio 1994, Planchais and Sinoquet 1998, Collet et al. 2002, Van Hess and Clerkx 2003) and physiological acclimation (Johnson et al. 1997, Aranda et al. 2002, Lemoine et al. 2002). Studies agree that beech seedling development positively responds to moderate opening of the overstory (Madsen and Larsen 1997, Tognetti et al. 1997, Topoliantz and Ponge 2000). Increment of light transmission to the soil influences belowground resources availability like water (Madsen 1994) or nitrogen (Denslow et al. 1998) and also favours the development of a dense understory vegetation (Balandier and Pauwels, 2002) which can severely increase competition with beech seedlings for belowground resources (Madsen 1995, Löf 2000, Welander and Ottosson 2000, Fotelli et al. 2001). Beech is also considered a drought-sensitive species (Backes and Leuschner 2000, Garcia-Plazaola and Becerril 2000) while several studies reported evidence of beech adaptation to water deficit (Cochard et al. 1999, Löf and Welander 2000, Schmull and Thomas 2000). Water competition interacts with nutrient uptake and can indirectly induce mineral deficits to the plants (Löf 2000). Therefore, it is often difficult to separate nutrient and water stress competition (Nambiar and Sands 1993). Although beech response to different light and water conditions taken separately is well documented, few studies have analysed the three main growth factors taken together (light, nutrients and water) and the interactions between them (Madsen 1995, Minotta and Pinzauti 1996). Because of the demonstrated shade-tolerant character of beech, research about beech

development in full-light conditions is still scarce, but in such conditions belowground competition induced by the surrounding vegetation seems to be the main factor constraining beech development (Löf 2000, Fotelli et al. 2001, Coll et al. 2003). The ability of woody plants to compete with herbs and grasses for natural resources has been pointed out as one of the main processes involved in vegetation succession in open lands or in forest gaps (Tilman 1988, Bazzaz 1996, Fotelli et al. 2001). Grasses (*Graminaceae*) are present particularly under full light conditions. Generally, they are considered as the most harmful for young tree growth through competition for water or nutrients (Frochot et al. 1986, Davis 1987) mainly due to their high root density in the superficial horizons (Casper and Jackson 1997, Coll et al. 2003) and to their particular root architecture which allow them to develop effective soil resource exploitation (Fitter et al. 1991). Since beech is considered a species with high acclimation capacity to contrasted environments, one could expect morphological and physiological responses to herbaceous competition as it has been reported for other species (Chaar et al. 1997, Mohammed et al. 1998).

The objectives of this study were: (1) to determine beech seedling requirements and growth under full light conditions; (2) to characterize water and nutrient competition between beech seedlings and a typical full light grass vegetation; and (3) to investigate whether beech seedlings develop different morphological and physiological patterns in relation to belowground competition.

Materials and methods

Experimental design

The experiment was set up at the CEMAGREF research institute of Aubière (45°45'N, 3°07' E, altitude: 394 m, central France). In October 2000, sixty 160 l containers were positioned in seven rows (eight or nine containers per row) spaced 1.5 m apart from each other with one meter distance between containers. Containers were filled with local soil with a loamy silt texture (pH = 6.2) and no mineral deficiency and were exposed to full light conditions. Thirty containers were maintained in bare soil and the other half was sown (October 2000) with a mixture of five grass species (0.4 g *Festuca rubra*, 0.789 g *Arrhenatherum elatius*, 0.018 g *Agrostis capillaris*, 0.062 g *Holcus lanatus* and 0.157 g *Dactylis glomerata* per container). These grasses are commonly found in semi-natural meadows of the area. The proportions of each grass species present in the sown mixture were chosen according to the natural abundance of each species recorded in a flora survey carried out during the previous summer (2000) in a recent abandoned meadow near the experimental site (Coll et al. 2003). The density sown in each container (33 Kg ha^{-1}) followed the current recommendations for grasses meadow implantation (Bodet et al. 1989).

In December 2000, 23 two-year-old bare rooted beech seedlings (52.8 ±7.9 cm stem height, 5.7 ±0.9 mm stem basal diameter) from a local tree nursery were planted in the recently sown containers and 23 other seedlings were planted in the bare soil containers. Thus, four different treatments were installed: beech grown with grass (BG, n=23), beech grown in bare-soil (B, n=23), grasses alone (G, n=7) and bare-soil with no vegetation nor tree (S, n=7). Containers were laid out in seven blocks, each containing three or four replicates of BG and B treatments and one replicate of S and G.

Grasses were regularly cut to a height of 20 cm to avoid any effect of light competition on beech seedlings. Measurements were carried out during the 2001 and 2002 growing seasons.

Beech seedling growth, architecture and biomass

Beech bud burst and shoot elongation dynamics were followed in eight seedlings per treatment (BG, B) from April to June in 2001 and 2002. Each bud was tagged and bud burst date and shoot elongation after bud burst were measured every week. When several growth flushes occurred, each polycyclic bud number and its shoot elongation were recorded. Beech stem basal diameter was measured for all the seedlings (n=46) every week from June to September. To account for daily diameter variations, three (2001) and eight (2002) seedlings per treatment in 2001 and 2002, respectively, were equipped with a linear variable differential transformer (LVDTs; model DF2.5, Solartron Metrology, Massy, France). The LVDT measurements were recorded as 10-min-means.

At the end of the first growing season (2001) six seedlings (one per block) per treatment were harvested and oven-dried at 70 °C for 96 h to determine leaf, shoot and root biomass. Forty fresh leaves were scanned and mean leaf area was computed using Winfolia software package (Régent Instruments Inc., Québec). The same protocol was applied to biomass measurements for the seedlings (n=26) harvested at the end of the second growing season (2002), although seventy leaves were used for mean leaf area calculations.

Soil water content (SWC) and seedling water status

Controlled irrigation with capillary tubes was used to supply containers with the same amount of water. Thus recorded differences in SWC were due to the treatment effect (grass, bare soil). In August 2002 a severe drought period was induced. The pots were well-watered and then were left without any water supply for 10 days.

Volumetric SWC (%) was measured weekly during both growing seasons from June to September to a depth of 20 cm with a tube TDR probe (Trime T3, IMKO, Ettlingen, Germany). Analyses were carried out in eight containers of each BG and B treatment and in two containers of S and G treatment. For each tube, the mean of three measurements in different directions was used for data analysis. During the drought-induced period (August 2002), SWC was measured every two days.

Midday xylem water pressure (P_x) of beech seedlings was measured with a Scholander chamber (Scholander et al. 1965) at different dates corresponding to contrasted levels of SWC and every two days during the drought-induced period.

Eight different seedlings were sampled and three leaves per tree were collected. The xylem water pressure (P_x) at the base of the leaf was measured on leaves enclosed previously for at least two hours in an air-proof aluminium foil bag (Cochard et al. 2002).

Starch and nitrogen content of beech seedlings

Shoot and root nitrogen content (mass basis, N, %) was measured with an elemental analyser (Carlo ERBA-1108; Carlo, Milan, Italy) in the six seedlings per treatment harvested at the end of the growing season. Starch content was also measured in the same compartments with a hexokinase, glucose-6-phosphate linked assay (Kunst et al. 1984) after hydrolysis with amyloglucosidase (Boehringer 1984).

Nitrogen fertilization and ^{15}N labelling

During the first growing season (2001) no fertilizer was added to the containers and whereas the second year 40mL (13th May) and 30 mL (7th June) of N-P-K (6-6-6) were applied to every container to get a non-limiting NPK starting point.

On 11 June and 14 August 2002, 500ml of water containing 30 mg of ^{15}N (10% excess in a solution of $^{15}NH_4{}^{15}NO_3$) was supplied to nine pots (three replicates of each BG, B, G treatment). Plastic walls were used to label the centre of the container only ($0.16m^2$) around the beech stem.

Respectively two and three weeks after the two labelling periods, the aboveground seedlings biomass was harvested and shoots and leaves were separated. Grass aerial biomass was collected by clipping the shoots (leaves and sheaths) which were then sorted by species.

Beech and grass root extraction from the soil was assessed by dividing the whole lump of soil in small subsamples which were then placed above a mesh of 2 mm and washed out with tap water (shower system). The roots were then collected and beech and grass roots were separated for subsequent analysis. All organs were oven-dried (60°C, 48h), finely milled and five to seven mg were weighed for ^{15}N analyses with a mass spectrometer (FISONS / ISOCHROM). The results were expressed in isotopic excess which corresponded to the difference between the sample abundance and the air abundance (0.3663%). The quantity of absorbed ^{15}N was also calculated.

In this study we did not measure the ^{15}N leaching. Nevertheless, the leaching risk was minimized during the ^{15}N labelling period since the 500 ml of labelled solution applied to the containers were entirely retained in the soil and containers were always watered below their soil water saturation level to avoid ^{15}N losses.

Leaf gas-exchange measurements

In August 2002, leaf gas-exchange was measured at three different dates (7, 13 and 19 August) during the drought induced-period corresponding to the beginning, middle and end of the period. Measurements of maximum steady-state net photosynthetic rates at light saturation (A_{max}), and associated stomatal conductance (g_{max}) were made in five different leaves

corresponding to five seedlings per treatment with a portable leaf chamber system (LI-6400, Li-Cor, Lincoln, NE, USA). For A_{max} calculation, the chamber was maintained at 1500 µmol $m^{-2}s^{-1}$ PAR and at 350 ppm CO_2. The air temperature and air-to-leaf water vapour pressure difference were kept constant (24-26 °C, 1-1.2 Pa Pa^{-1}). Equilibrium values generally reached within 20-30 min were considered for subsequent analysis. After measurements, leaves were collected, scanned and their leaf area determined with Winfolia software package (Régent Instruments Inc., Québec). They were oven-dried and nitrogen content was measured with an elemental analyser (Carlo ERBA-1108; Carlo, Milan, Italy). Leaf N was expressed per unit leaf area (N_s, g m^{-2}).

Data analysis

Least significant distance (LSD) method was used to evaluate growth and morphological differences between seedlings for the different treatments. Diameter and height growth rates were calculated for 2001 and 2002 by dividing the seasonal increment by the initial value of each growing season. LSD test on percentage data was applied after arcsin square root transformation of the values to meet the conditions of normality and homoscedasticity.

To take into account the possible different initial sizes of the seedlings, relative diameter increment (RDI, day^{-1}) was used. RDI was calculated from:

$$RDI = 1/D_1 \times [(D_2-D_1) / (t_2 - t_1)] \quad (1)$$

where t_1 and t_2 are two consecutive measurement dates. For beech seedling stem diameter, D_1 is the basal diameter at t_1 and D_2 is the basal diameter at t_2.

Volume SWC associated to each RDI period is given by the mean value between SWC at t_1 and SWC at t_2.

Treatment differences in A_{max}, g_{max} and in the ratio of A_{max}: N_s in relation to P_x were assessed by analysis of covariance (with treatment as the main factor and P_x as the covariate) after logarithmic transformation of the dependent variable.

As there was no statistical block effect whatever the considered variable, each container was considered as a pseudo-replication in the subsequent analyses.

Data were analysed using Statgraphics Plus 5.1 (Statistical Graphics Corp.) software and unless otherwise indicated, probability values <0.05 were considered significant.

Results

Beech seedling development and morphology

Beech stem diameter growth differed significantly ($p<0.05$) between treatments (Table 1). In the case of beech seedlings grown without grasses (B) diameter increments were 1.6 and 2.8 times greater for the 1st and 2nd growing season, respectively, than the seedlings grown with grasses (BG). Height growth only varied significantly during the second growing season (data not shown). The B seedlings increased their initial height by 87% while BG seedlings scarcely grew (1%).

Differences in beech morphology and biomass allocation were found between treatments (Table 1). Seedling leaf area and leaf number (data not shown) were significantly higher in the B treatment in 2002 but not during 2001. Bud burst rate did not vary between treatments, but strong differences in total shoot elongation were found in 2002, since 99% of the growth units corresponding to the BG seedlings did not reach shoots longer than 1 cm.

At the end of the experiment, the biomasses of stem and root were 7.8 and 5.3 times greater in the B beech seedlings than in the BG ones, respectively.

No difference in biomass allocation between treatments was found at the end of the first growing season. However, the root-shoot biomass ratio increased significantly in the BG seedlings during the second growing season, and significant differences were found between treatments (0.74 *vs* 1.24 in the B and BG treatments respectively).

Beech seedling growth response to soil water content

Containers were always supplied with the same amount of water during the whole of the experiment, but strong SWC differences ($p<0.05$) were found between B and BG treatments during the two growing seasons (figure 1). These differences were greater during the second

1 growing season despite containers receiving more water that year (see bare soil curve). No
2 significant difference was found between the SWC of the BG and G containers.
3 There was a positive correlation between the beech relative diameter increment measured
4 with the LVDT sensors and the SWC of the container (figure 2). This relationship was more
5 consistent the second year of the experiment and the slope was higher than the first year.
6 Beech xylem water pressure (P_x) was negatively correlated to SWC values (figure 3) and
7 mean P_x ranged from -0.5 to -2.4 MPa.
8
9 *Water and nitrogen effect on leaf gas exchange of beech*
10 In August 2002, beech seedlings reduced their maximum stomatal conductance (g_{max}) in
11 response to P_x decrease (figure 4a). Differences between treatments were found (table 2) and
12 for the same P_x level B beech seedlings exhibited higher values of g_{max} than BG seedlings.
13 The same pattern was found when A_{max} was related to P_x (figure 4b, table 2) when BG
14 seedlings presented significantly lower A_{max} values for a same P_x level.
15 Leaf nitrogen analyses of B seedlings revealed significantly higher N content per leaf area
16 (N_s) values than BG seedling leaves (0.017 *vs* 0.013 g cm^{-2}). When the ratio between Amax:
17 N_s is related to P_x , no significant differences (p<0.05) between treatments were observed
18 (figure 4c, table 2). Nevertheless, the *p*-value (0.059) was close to the significance level used
19 for the analysis of covariance, mainly due to higher Amax : Ns found in B seedlings under
20 high water deficit conditions (P_x lower than -2.5 MPa).
21
22 *Beech seedlings nitrogen and starch content at the end of the first growing season*
23 At the end of the first growing season, significantly higher (p<0.05) shoot and root N content
24 was found in beech growing in bare soil when compared with beech growing with grass (table
25 3). Comparison of root and shoot starch content between treatments did not show significant

differences. Nitrogen and starch were mainly located in the root system except for the BG treatment with high N content in shoot system.

Distribution of ^{15}N and N between beech and grass

Results from the ^{15}N labelling (figure 5) showed differences between treatments and dates. In the B treatment, beech ^{15}N excess was higher ($p<0.05$) in September than in June for all the plant compartments, while it was the opposite for the BG treatment. In June, the seedlings of the BG treatment incorporated 0.278mg of ^{15}N (plant level) that corresponded to values twice as high as those of the B seedlings. In September, BG took up less ^{15}N (0.175mg) while the ^{15}N uptake of the B seedlings increased significantly (3.915 mg).

The distribution of ^{15}N between compartments was unaffected by the presence of grasses. In the case of the grasses, no significant differences between-dates were observed in ^{15}N excess and ^{15}N mg. The presence of beech did not affect the labelling values (data not shown). Most of the ^{15}N was allocated to the leaves, 78% and 69% in June and September, respectively. Moreover, the grasses took up between 40-46% of the ^{15}N supply whereas beech incorporated less than 1% in BG and B (June) and about 13% in September in the B treatment. In September significantly lower N content (N%) was found for the shoot compartment in B seedlings while leaves and roots did not present significant differences between dates. Seedlings from the BG treatment showed significantly lower N% in their leaves in September when compared to June, but no differences were found on root and shoot N% between both dates. When comparing N content values between treatments, results always showed lower N% values in BG beech than in B beech for all compartments except for leaves in the first labelling date (June).

Discussion

Effect of belowground competition induced by grasses

In the absence of grass competition, beech seedlings under full light conditions experienced high growth levels (table 1). In a parallel experiment carried out in natural conditions with the same plant material high levels of beech development in seedlings planted in a weeded full-light plot were found when compared with different shelterwood conditions (Coll et al. 2003). Nevertheless, this pattern would be restricted to areas with no limiting soil water availability as reported by Madsen (1994).

Beech seedlings exposed for two years to belowground competition induced by grasses (light competition was minimized by maintaining grass under tree leaves) presented strong reductions of mainly growth parameters (stem diameter, height growth, biomass) (table 1, figure 2). Results agree with numerous studies which reported the negative effect of vegetation competition on seedling development (Sands and Nambiar 1984, Cole and Newton 1987, Collet et al. 1996). In both growing seasons, grass presence greatly reduced soil water content, and thus water availability for beech seedlings (figure 1) proving the high grass (*Graminaceae*) competitiveness for belowground resources as reported in other studies (Frochot et al. 1986, Davies 1987). In the present study, the decrease of seedling diameter increment was mainly related to a decrease of soil water content in the presence of grass underlying the effect of water competition by grasses on seedling development (Picon-Cochard et al. 2001). This relationship was less consistent during the first growing season probably due to transplanting shock (Kozlowski and Davies 1975, Jobidon et al. 1998) acting for both treatments (BG and B). Low SWC values caused decreases on seedling xylem water pressure and consequent stomatal closure occurred (figures 3, 4) (Aranda et al. 2000, Backes and Leuschner 2000) that could prevent catastrophic development of xylem embolism (Tyree

and Sperry 1988, Cochard et al. 2002). Moreover such stomatal closure reduces CO_2 flux to the chloroplasts and therefore net assimilation rates. In this experiment, beech growing with grasses presented lower g_{max} for the same P_x level than beech growing in bare soil (figure 4a). Low g_{max} on BG seedlings could reflect signs of beech acclimation to soil water deficit through faster stomatal closure, thus limiting water loss under water deficit conditions. Beech acclimation to high evaporative demand in terms of lower beech xylem vulnerability has been reported by Cochard et al. (1999) and Lemoine et al. (2002), but this trend was not related with stomatal dynamics. The lower maximum photosynthetic rate values on BG seedlings when compared with B ones for the same P_x level (figure 4b), might be explained by significantly higher leaf N_s content on seedlings from the B treatment, since differences between treatments disappeared when A_{max} was divided by N_s (figure 4c). The relation between A_{max} and the leaf nitrogen content is well known (Chapin et al. 1987) and is mainly due to the fact that nitrogen is involved in ribulose-1.5-bisphosphate carboxylase-oxygenase (Rubisco) regeneration, the primary CO_2-fixing enzyme (Evans 1983). The relationship between A_{max} and N_s decreased with increasing water deficit probably because, in such conditions, A_{max} should mainly be limited by stomatal conductance reductions.

Nitrogen analysis and ^{15}N labelling clearly put into evidence nitrogen competition induced by grasses (figure 5). For two different dates, corresponding to (1) the end of seedling leaf expansion and (2) after a severe drought period, grasses from the BG treatment took up more than 97% of the total ^{15}N incorporated in the plants (beech + grasses) for the same SWC value. The asymmetric ^{15}N distribution between seedlings and grasses could be explained by a higher interception efficiency combined with higher N use efficiency of the grasses (Elliot and White 1987, Campbell et al. 1994, Coll et al. 2003). The grass root system is denser than that of the beech, and is mainly composed of thin roots (diameter < 0.5mm) associated with high absorption capacity (Robinson et al. 1991). According to Fitter et al. (2001) a main root

trait associated with mineral absorption is the specific root length (SLR). In the case of grasses SLR can reach 700 m g^{-1}DW (Atkinson 2000) while that of beech hardly reaches 20 m g^{-1}DW in natural condition measurements (Hendricks and Bianchi 1995, Curt and Prévosto 2003). Even if all the roots are not active for N absorption, only around 10% according to Robinson et al. (1991), the grasses were more efficient at absorbing nutriments than beech seedlings. Moreover, a beech seedling growing with grass presented a poorly developed root system, thus limiting its possibility to intercept nutrients. Nitrogen absorption by roots is also closely related to soil water content (Barber 1962), but in the case of mobile ions such as nitrogen, the spatial distribution of the root system could be more limiting for absorption in soil drought conditions (Caldwell and Richards 1986). This study showed evidence of both water and N competition, but the experimental design did not allow us to assess the effect of each resource separately on beech growth. Nitrogen uptake in the B seedlings was higher in September than in June. Fine root growth and thus the development of a more efficient root system for belowground resource absorption (Espeleta and Donovan 2002) has been reported to peak in July-August for beech species in temperate conditions (Riedacker 1981, Büttner and Leuschner, 1994), consequently after the first labelling date in our study. This could probably explain the better N uptake of the B seedlings in September. Beech growing with grass did not present differences in N uptake between both dates, but uptake levels remained very low at both dates. Thus these seedlings accumulated less N content in roots and stems at the end of the summer, while N leaf content fell to 1.58%, which corresponded to a critical value for beech species (Bonneau 1988).

Leaf N content in the BG seedlings was similar to the B seedlings at the beginning of the growing season, but the mean leaf area was considerably reduced for the BG seedlings (tables 1 and 2). This pattern is probably related to the spring remobilization of stored N, which has been pointed out as the main N source for spring leaf growth whatever the soil N availability

at this period (Millard 1996). As stored nitrogen at the end of the first growing season was lower in the BG seedlings (table 3), they had probably maintained high N concentration levels in new growing leaves by reducing their size since these organs are stronger N sinks allowing a high photosynthesis capacity to be maintained. Leaf size reductions induced by low nitrogen availability have also been observed for other tree species as *Betula pendula* (Paakkonen and Holopainen 1995) or *Liquidambar styraciflua* (Kuers and Steinbeck 1998).

Finally, in this study the use of containers forced grass and beech roots to develop in a limited exploitable soil volume. In natural conditions, spatial partitioning of soil resources between trees and grasses has been observed (Burch et al. 1997, Casper and Jackson 1997, Dawson et al. 2001) with trees commonly presenting deeper rooting than grasses. However, our study focused on the early stages of tree development which are usually characterized by severe root competition in the uppermost soil horizons between the tree seedlings and the ground vegetation.

Consequences of grass competition on seedling morphology and biomass

During the second growing season grasses induced significant differences on seedling architecture. Beech growing with grass exhibited strongly reduced shoot elongation, and up to 99% of buds elongated less than 1 cm ("short growth units.", Nicolini and Chanson 1999). In this experiment, the lower SWC induced by grass in 2001 may be the main factor inducing low shoot elongation in 2002 as previously reported by Löf and Welander (2000) and Chaar et al. (1997). Recently, a positive correlation between the primary meristematic activity and the beech water status has been found (Cochard, personal communication). This correlation could explain the relationship between previous-year drought and current shoot elongation.

Grass also induced lower seedling leaf biomass by both reducing leaf number (probably related to lower growth unit length) and leaf size. Biomass differences in seedlings could also

result from differences in starch storage between treatments (Gansert and Sprick 1998), although in the present experiment starch concentration was unaffected (table 2) the quantity of stored starch should be different between treatments due to differential biomass accumulations (table 1).

Finally, differences in growth allocation were found between treatments, and beech growing with grass presented higher root to shoot ratio than those growing in bare soil. This result agreed with other studies which reported higher carbon allocation to roots under belowground competition (Van Hess 1997, Shipley and Meziane 2002) and emphasized morphological acclimation of beech seedlings to water and nitrogen competition.

Conclusion

Although beech is considered a shade-tolerant species, beech seedlings presented higher diameter and height growth rates in full light conditions in the absence of belowground competition. Grasses have induced high water and nutrient competition with consequent reduction of beech growth by limiting photosynthesis rate and thus root absorption.

Some signs of leaf physiological acclimation to water competition (stomatal closure) have been observed in the seedlings with grass.

Grass induced marked differences on seedling morphology and strong reductions of bud elongation, stem diameter and leaf area were observed. This is likely to have been caused by previous-year lower SWC in seedlings from BG treatment.

We conclude that high sensitivity to water and nutrient competition with typical full light grass species seems to be the main obstacle for beech development in grassland in the absence of a shelterwood protection.

Acknowledgements

We thank A. Marquier and F. Landré for their valuable technical work during measurements and B. Prévosto, G. Kunstler and two anonymous reviewers for helpful comments and suggestions. The PhD grant to L.C. was funded by CEMAGREF and the Auvergne Region. The study was partly funded by the DRAF Auvergne (FNADT) and the Auvergne Region.

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Table 1. Growth and main morphological characteristics (mean, ± S.E.) of beech seedlings grown in containers without grass (B) and with a sowed mixture of grass species (BG) at the end of two growing season (2001 and 2002). For each year and growth variable, (**) indicates significant differences between treatments (p<0.01) and (ns) no significant differences (p<0.05) while n is the number of replicates par treatment for each variable.

	Cumulated shoot elongation (cm)		**% short growth units (<1 cm)**		**Diameter growth (%)**		**Leaf biomass (g)**		**Stem biomass (g)**		**Root biomass (g)**		**Area per leaf (cm^2)**		**Root : shoot ratio**	
year	2001	2002	2001	2002	2001	2002	2001	2002	2001	2002	2001	2002	2001	2002	2001	2002
	(n=8)	*(n=8)*	*(n=8)*	*(n=8)*	*(n=22)*	*(n=13)*	*(n=6)*	*(n=13)*	*(n=6)*	*(n=13)*	*(n=6)*	*(n=13)*	*(n=40)*	*(n=70)*	*(n=6)*	*(n=13)*
	ns	**	ns	**	**	**	ns	**	ns	**	ns	**	ns	**	ns	**
B	71.9	621.0	48.6	51.6	61.9	96.4	7.50	44.09	19.98	147.20	21.87	144.01	9.6	14.1	0.77	0.74
	(± 9.9)	(± 44.4)	(± 5.4)	(± 5.6)	(± 3.5)	(± 5.5)	(± 0.62)	(± 4.41)	(± 2.76)	(± 13.73)	(± 3.44)	(± 15.93)	(± 0.4)	(± 0.5)	(± 0.04)	(± 0.05)
BG	78.3	6.6	63.8	99.6	37.3	34.2	6.01	2.66	10.55	19.50	14.34	28.19	8.6	8.1	0.92	1.24
	(± 8.0)	(± 1.0)	(± 3.3)	(± 0.3)	(± 1.6)	(± 3.1)	(± 1.12)	(± 0.39)	(± 2.62)	(± 1.96)	(± 4.71)	(± 3.55)	(± 0.4)	(± 0.3)	(± 0.10)	(± 0.06)

Table 2. Summary of the analysis of covariance for g_{max}, A_{max} and the ratio A_{max}:N_s with treatment (BG or B) as main factor and P_x as covariate.

	g_{max}			A_{max}			A_{max} : N_s		
	df	F	*p-value*	*df*	F	*p-value*	*df*	F	*p-value*
Treatment	1	3.89	0.0015	1	20.82	0.0001	1	12.63	0.0592
P_x	1	155.59	0.0000	1	148.83	0.0000	1	108.47	0.0000

Table 3. Stem and root nitrogen and starch contents (n=6, mean, ± S.E.) at the end of the first growing season (2001) for the seedlings grown without (B) or with grasses (BG). For each organ, significant differences between treatments are noted with different letters (p<0.05).

	Nitrogen content (%)		Starch content (mg g^{-1})	
Treatment	**Shoot**	**Root**	**Shoot**	**Root**
B	1.28 (± 0.06) a	1.67 (±0.03) a	21.4 (± 1.8) a	95.7 (± 6.7) a
BG	0.76 (± 0.06) b	0.60 (±0.04) b	22.4 (± 4.4) a	141.2 (± 40.1) a

Figure 1. Time course of the relative soil water content (mean ± standard error) (see M & M section) as measured by TDR probe during the growing season 2001 and 2002 for the upper (0-20 cm) soil horizon. Dark circles correspond to beech seedlings grown without grasses (B) and white circles to beech growing with grasses (BG) treatments. Solid lines represent the bare-soil containers (S) and dashed lines the grass containers (G).

Figure 2. Relationship between the relative diameter increment (RDI) measured with LVDT sensors and the mean volume soil water content (SWC) between two consecutive measurement dates. Each LVDT values is the mean of three (circle, 2001) and eight seedlings (square, 2002) per treatment (dark corresponding to B, white corresponding to BG), while each SWC values is the mean of eight measurements. Solid lines are fitted regressions (2001 : $y = -0.002 + 0.0008 \ln x$, $R^2 = 0.46$; 2002: $y = -0.0056 + 0.002 \ln x$, $R^2 = 0.79$)

Figure 3. Relationship between the volume soil water content given by TDR probe (0-20 cm) (n=8 per treatment) and the xylem water pressure (n=8 seedlings per treatment) at four different dates of the 2nd growing season. Dark circles correspond to beech seedlings grown without grasses (B) and white circles to beech growing grasses (BG) treatments Vertical and horizontal bars correspond to SE.

Figure 4. Relationship between g_{max}, A_{max} and A_{max}: N_s with the xylem water pressure (P_x) during the drought-induced period of beech seedlings grown with grasses (BG) and without grasses (B). Measurements were performed in 7, 13 and 19 August 2002.

Figure 5. Beech seedling leaves, shoot and root ^{15}N isotopic excess (%^{15}N), absorbed ^{15}N (Q ^{15}N, mg) and nitrogen content (%N) for B and BG seedlings at the end of the two labelling dates. In the case of grasses leaves and root %^{15}N, Q ^{15}N and %N were also expressed.

Figure 1

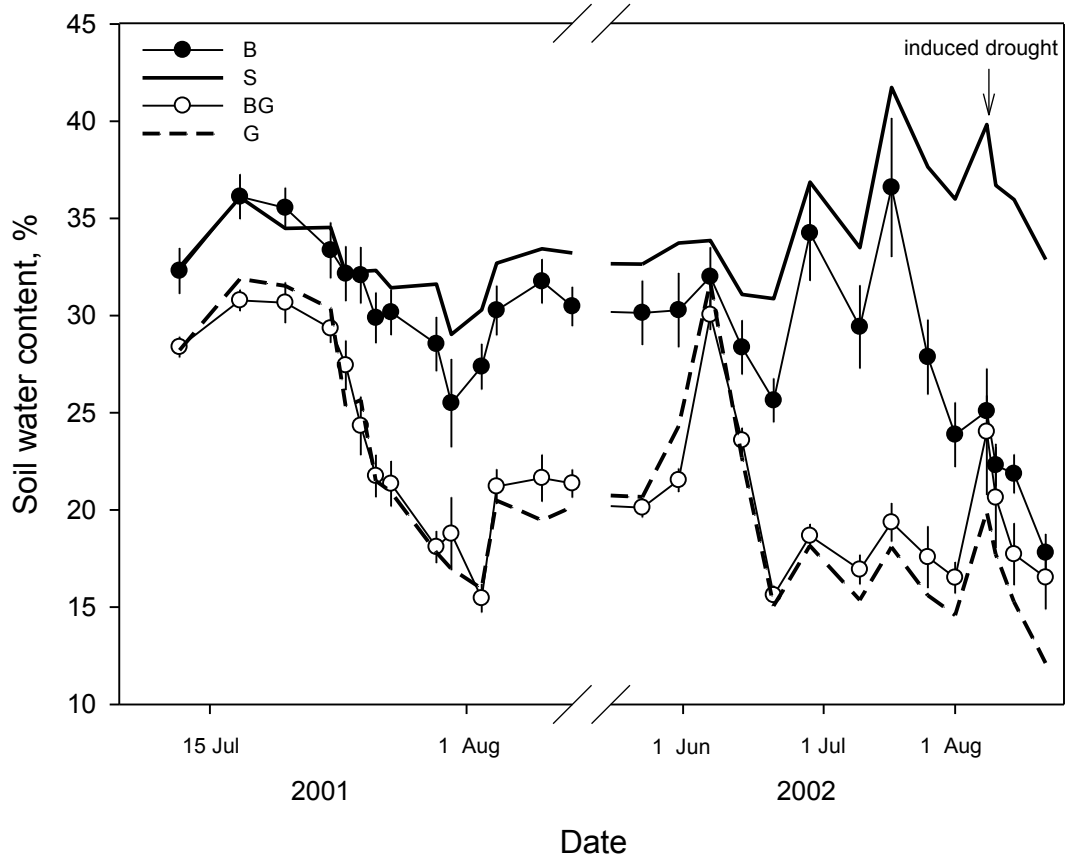

B
S
BG
G
induced drought
Soil water content, %
45
40
35
30
25
20
15
10
15 Jul
1 Aug
1 Jun
1 Jul
1 Aug
2001
2002
Date

Figure 2

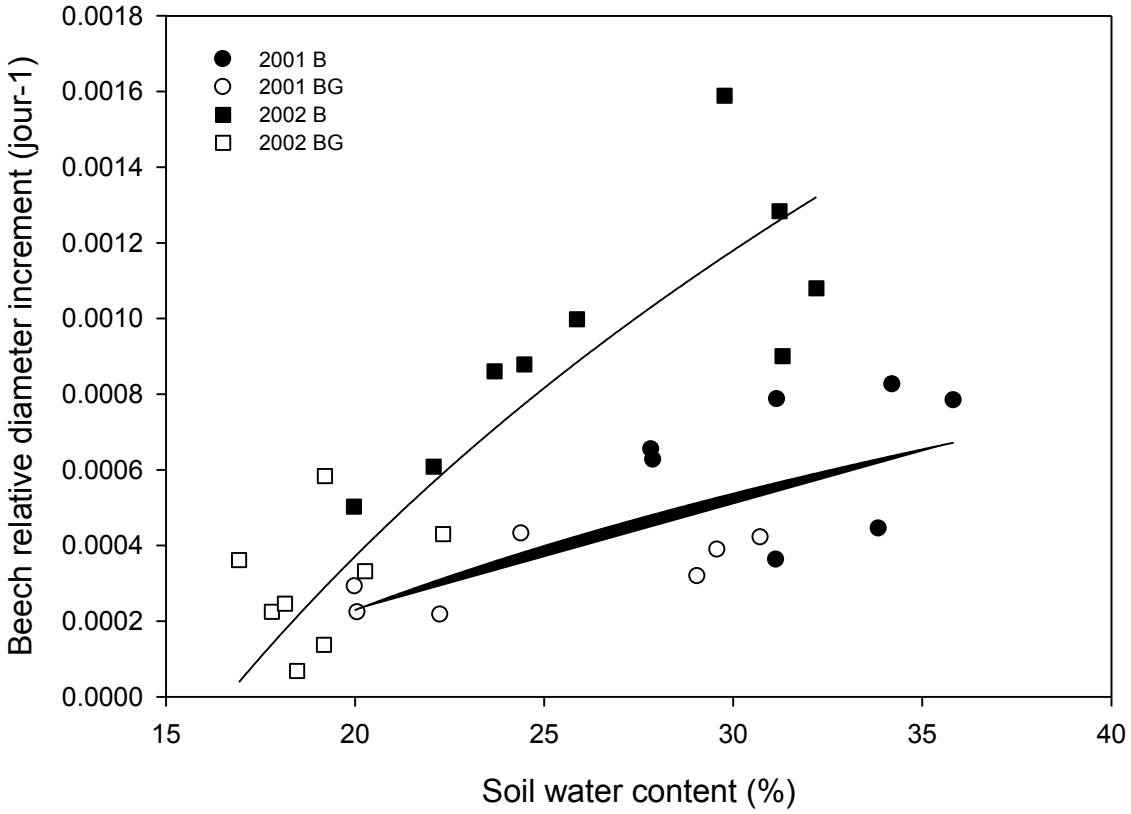

Beech relative diameter increment (jour-1)
0.0018
0.0016
0.0014
0.0012
0.0010
0.0008
0.0006
0.0004
0.0002
0.0000
2001 B
2001 BG
2002 B
2002 BG
15
20
25
30
35
40
Soil water content (%)

Figure 3

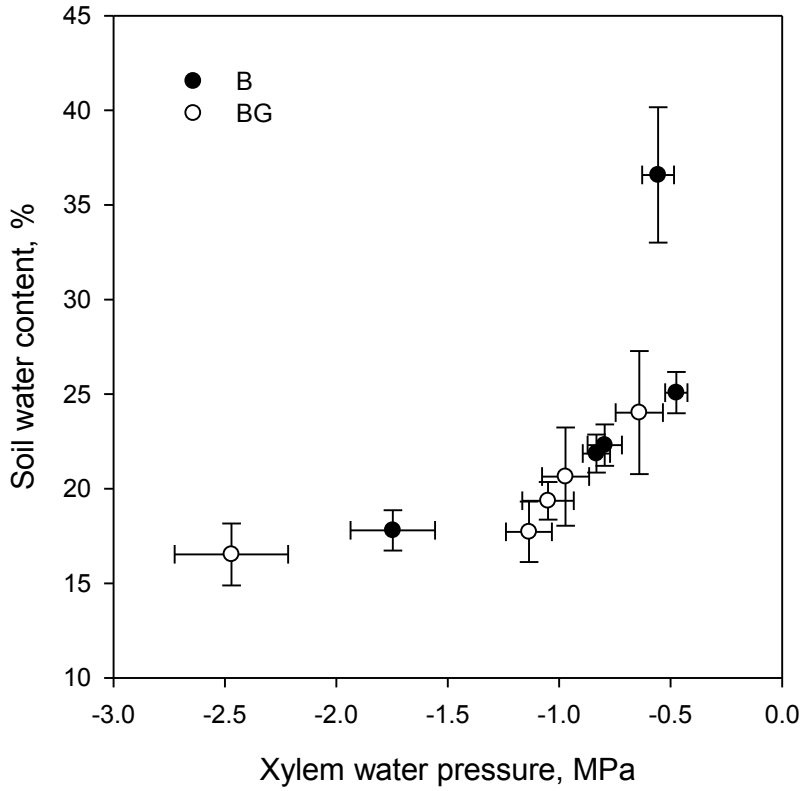

B
BG
Soil water content, %
Xylem water pressure, MPa
45
40
35
30
25
20
15
10
-3.0
-2.5
-2.0
-1.5
-1.0
-0.5
0.0

Figure 4

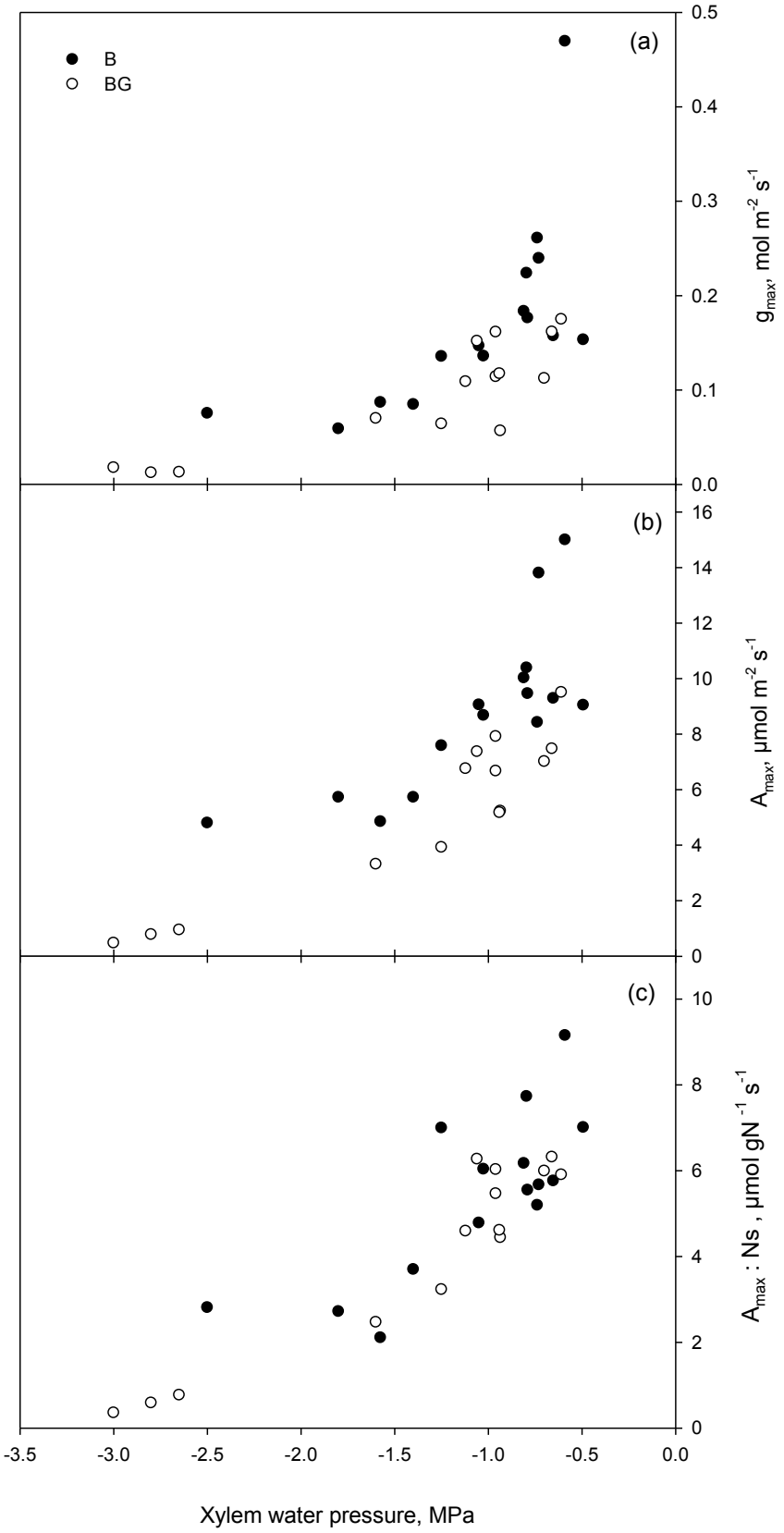
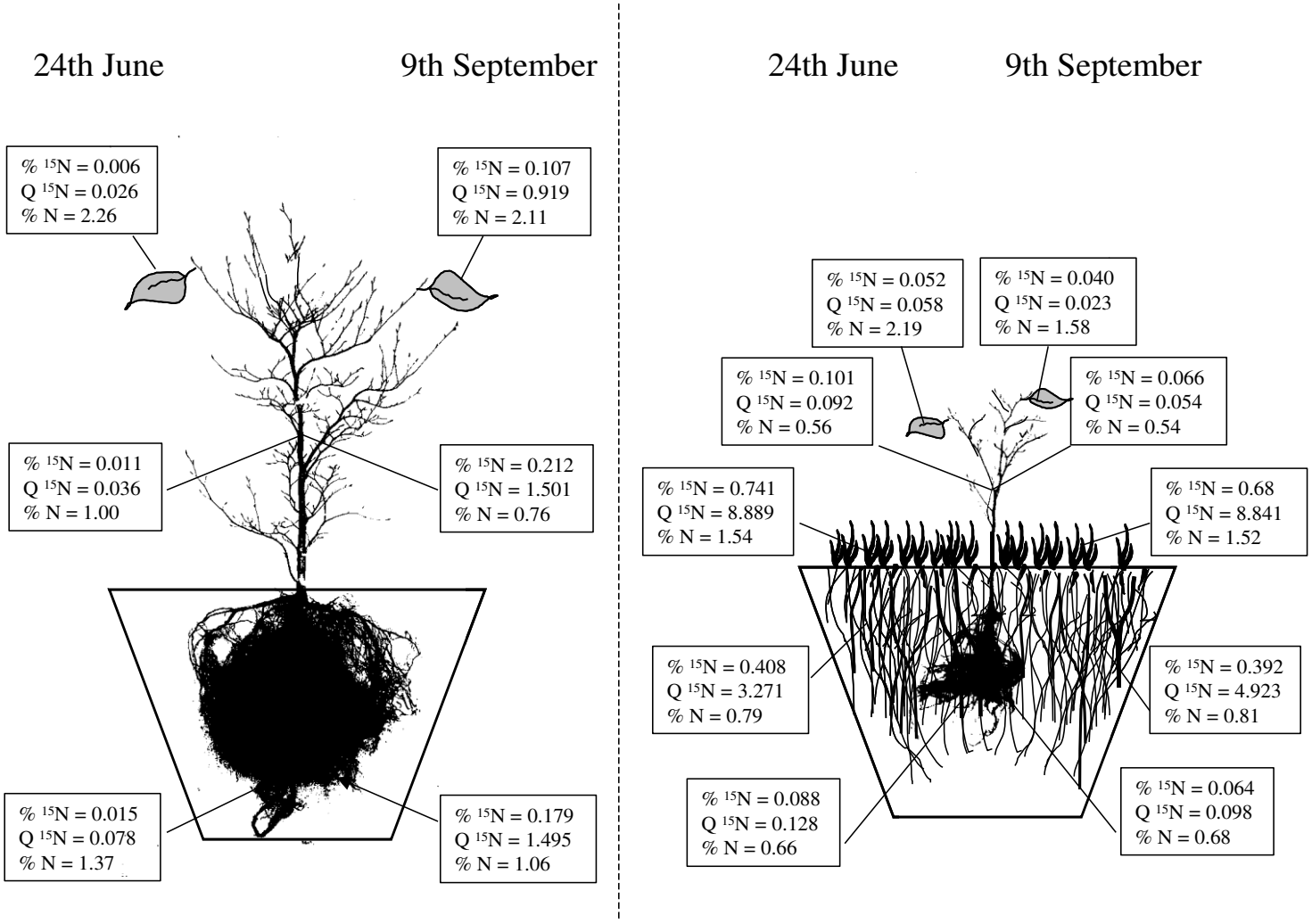

B
BG
(a)
(b)
(c)
g_{max}, mol m^{-2} s^{-1}
A_{max}, µmol m^{-2} s^{-1}
A_{max} : Ns , µmol gN^{-1} s^{-1}
Xylem water pressure, MPa

Figure 5


24th June
9th September
% 15N = 0.006
Q 15N = 0.026
% N = 2.26
% 15N = 0.107
Q 15N = 0.919
% N = 2.11
% 15N = 0.011
Q 15N = 0.036
% N = 1.00
% 15N = 0.212
Q 15N = 1.501
% N = 0.76
% 15N = 0.015
Q 15N = 0.078
% N = 1.37
% 15N = 0.179
Q 15N = 1.495
% N = 1.06
24th June
9th September
% 15N = 0.052
Q 15N = 0.058
% N = 2.19
% 15N = 0.040
Q 15N = 0.023
% N = 1.58
% 15N = 0.101
Q 15N = 0.092
% N = 0.56
% 15N = 0.066
Q 15N = 0.054
% N = 0.54
% 15N = 0.741
Q 15N = 8.889
% N = 1.54
% 15N = 0.68
Q 15N = 8.841
% N = 1.52
% 15N = 0.408
Q 15N = 3.271
% N = 0.79
% 15N = 0.392
Q 15N = 4.923
% N = 0.81
% 15N = 0.088
Q 15N = 0.128
% N = 0.66
% 15N = 0.064
Q 15N = 0.098
% N = 0.68